# THE ENDOCRINE SYSTEM OF *TYPHLOMOLGE RATHBUNI.*

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The endocrine system of *Typhlomolge rathbuni*, the blind Texan cave salamander, has been a matter of controversy for some time. In order to clarify some of the points under discussion, I have made serial sections of the region of the lower jaw, throat, and heart of 5 specimens, and the entire head of 2 specimens of this animal. The specimens were captured in Texas, as described in a previous article.[1] Six of them died during the trip from Texas to New York, one after 14 months of captivity in the laboratory. Since they are preserved after death, only the anatomical features of the various organs can be studied. A histological study must be postponed until suitable material can be secured.

## The Thyroid.

Emerson[2] was the first one to call attention to the possible absence of the thyroid gland in *Typhlomolge*. In 1905 she examined sections through the head of one specimen and was unable to find a thyroid. At the time Emerson published her paper the interest in the endocrine system of amphibians was very slight and her paper remained unknown to most biologists. In several of my papers on the thyroid function of salamanders I have called attention to Miss Emerson's interesting findings, which I had recognized to be correct. Soon after my return from Texas in 1916, I sectioned one of the *Typhlomolge* captured there and found the thyroid absent.

But at the 1921 Christmas meeting of the Anatomists, Swingle, apparently unacquainted with the literature on these facts spoke of the thyroid of *Typhlomolge* as a matter of fact and claimed to have isolated and observed this organ under the microscope.

[1] Uhlenhuth, E., *Biol. Bull.*, 1921, XL., 73.

[2] Emerson, E. T., *Proc. Soc. Nat. History, Boston*, 1905, XXXII., 43.

Although I mentioned my own findings, Swingle's very definite claims made the correctness of my observations doubtful, and even Doctor Wilder, from whose laboratory Emerson's paper was published, was ready to admit the possibility of an oversight on the part of Miss Emerson.

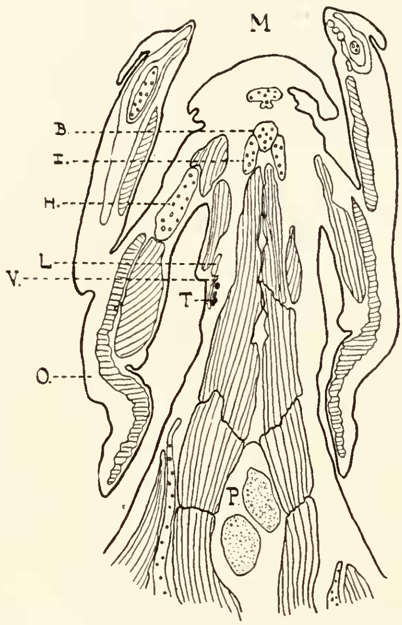
Immediately after my return from this meeting, I made sections of the 7 specimens discussed in this paper, and upon examination of the first one I was convinced that I was correct. In response to a letter in which Swingle admitted that the organ which he had claimed to be a thyroid was another vesicular organ, I communicated my new observations to Mr. Swingle. Neither this communication nor the incident at the Anatomists' meeting has been mentioned in an account recently published by Mr. Swingle in which he[1] states that 3 specimens examined by him possessed no thyroid.

The examination of the 7 specimens, together with previous findings, shows that while some specimens of *Typhlomolge* are without even vestiges of the thyroid, others possess epithelial structures, evidently undifferentiated thyroid rudiments whose development was inhibited by an unknown factor.

Before describing these rudiments, the location of a normal thyroid may be briefly referred to. For comparison I shall use the thyroid of the Ambystomidæ, which may be called representative of a normal salamander thyroid. In the Ambystomidæ, the median thryroid rudiment splits up into two epithelial cell masses, which migrate in a posterior, lateral and largely ventral direction until, in *Ambystoma opacum*, they are closely attached to a large lymphatic space (Fig. 1). This space is located in the interstitial space formed by the muscles which surround the first gill arch, ventral and median to the epibranchial of the first gill arch. In other species the thyroids may be located slightly further posterior, but always the lymphatic space where it is in touch with the thyroid is adjacent also to the anterior cardinal veins. At the site where the ventral end of the thyroid is attached to the lymph space, the anterior cardinal vein comes into close contact with this space, leaving there the thyroid, at its ventral and posterior end, as a large vessel into which collects the blood from the interfollicular rete of the thyroid.

[1] Swingle, W. W., *Jour. Exp. Zoöl.*, 1922, XXXVI., 397.

In addition to the main portions of the thyroid, most specimens possess accessory thyroids. These develop from small cell groups which during migration become detached from the main portions and thus mark the path along which the main portions




In reproduction figures 1 to 8 have been reduced by one third, figures 9 and 10 by slightly more than one third.

FIG. 1. Location of main portion of right thyroid in an advanced larva (59.2 mm. total length) of *Ambystoma opacum; I*, first gill arch; *B*, first basibranchial; *H*, hyoid; *L*, lymph space; *M*, cavity of mouth; *O*, operculum; *P*, pericard; *T*, thyroid; *V*, anterior cardinal vein.

migrate. Their location varies greatly. They are located usually anterior and may be either ventral or dorsal or, in case of several accessories, both ventral and dorsal to the main portions. Or they may be at one level with the main portions. They consist either of one or several median rudiments located in the median interstitium of the muscles ventral to the basibranchials of the visceral skeleton (genio-hyoideus) or of two lateral portions, one on each side, which either are attached to the sides of the basibranchials or are located in the interstitia of the muscles lateral and ventral to the basibranchials. In some Ambystomidæ

(*A. tigrinum*) the lateral portions may develop into normal thyroids of considerable size.

The thyroid rudiments of *Typhlomolge* occupy a position closely resembling the location of the various thyroid portions of the Ambystomidæ.

*Typhlomolge* 1, a sex-mature animal of 111 mm. total length and 58.2 mm. body length, does not possess even vestiges of a thyroid. The region of the lower jaw, throat, and heart was sectioned into a complete series; no section is missing. The anterior cardinal vein was followed in its entire course, the visceral cartilages and muscles were carefully searched through, but no traces of a thyroid could be found.

*Typhlomolge* 3, a small, apparently young, animal of 57.7 mm. total length and 32.6 mm. body length possesses a median thyroid rudiment. It is entirely detached from the pharyngeal epithelium and partly imbedded into the muscles just ventral to the basibranchial (genio-hyoideus). It is located between the attachments, to the basibranchial, of the hyoids and first gill arches and just anterior to the latter ones. The tissues are not well preserved, but the rudiment is seen to consist of several vesicles possessing epithelial walls and containing no colloid. No other epithelial structures were found, although the anterior cardinal vein was searched in its entire course down to the *Ductus cuvieri*, and the lymphatic space, the muscles, and cartilages of the visceral skeleton were carefully inspected.

In *Typhlomolge* 7, an animal of 75. 6 mm. total length and 43.1 mm. body length, a median rudiment is attached to the ventral surface of the muscles just ventral to the basibranchial, about in the middle between the attachments of the hyoid and first gill arches (Fig. 2). It consists of a single solid cell mass of epithelial structure (Fig. 3). In the center a network-like structure is noticed, produced very likely by the cell walls of the clear inner cell ends; the same structure is frequently found in tangential sections through the walls of the follicles of normal thyroids. No colloid is contained in this rudiment. In addition to the median rudiment, two lateral rudiments are present, one on each side. The anterior ends of these rudiments are located near the connection between ceratobranchial and epibranchial of the first gill arch and median to this arch (Fig. 4). They extend in a posterior direction; the posterior end approaches closely the wall of the lymph space, but does not come in contact with it. It is

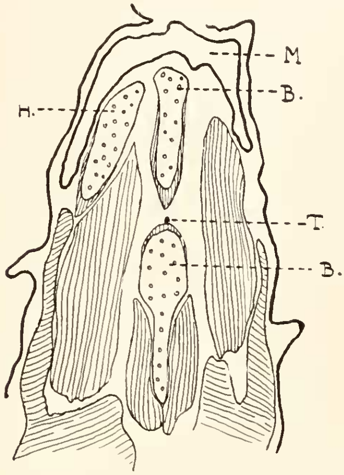



FIG. 2. Location of median thyroid rudiment in *Typhlomolge* 7. *B*, basibranchial; *H*, hyoid; *M*, cavity of mouth; *T*, thyroid rudiment.

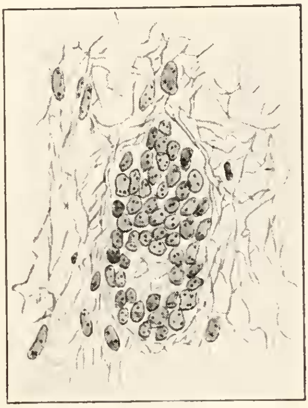


FIG. 3. Median thyroid rudiment of *Typhlomolge* 7. × 340.

evident from this account that the lateral rudiments of *Typhlomolge* occupy a location similar to that of the main portions of the thyroid in *Ambystoma opacum*. They are, however, located further anterior, as if they had stopped migrating before attaining the definite position. Moreover, the place where the anterior cardinal vein passes the lymph space is located considerably more ventral; therefore, the lateral rudiments are nowhere in contact with this vein. It is indeed impossible to see any vessels supplying the thyroid rudiment;

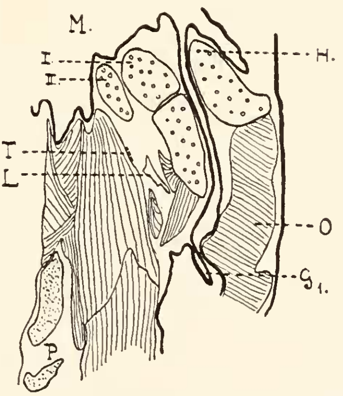



FIG. 4. Location of lateral thyroid rudiment of *Typhlomolge* 7. I., first gill arch; II., second gill arch; $G_1$, plate of first gill; *H*, hyoid; *L*, lymph space; *M*, cavity of mouth; *O*, operculum; *T*, thyroid rudiment.

if there are any they must be very small. The lateral rudiments consist of a series of tiny epithelial cell masses; some of them are solid, others are hollow, but none of them contain colloid.

In *Typhlomolge* 6, the smallest and probably youngest animal (56.0 mm. total length and 32.0 mm. body length), no median but one lateral rudiment on each side is present. They consist of a series of cell masses (Fig. 5), some of which are solid while others contain a lumen. Colloid is absent in all of them. The location is similar to that of the lateral rudiments of the previous specimen. In particular they do not touch the lymph space and are situated dorsal to the place where the anterior cardinal vein comes into contact with this space. They are without a blood supply resembling that of a thyroid.

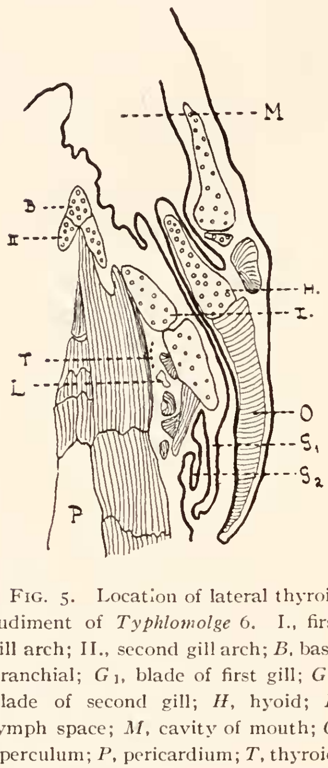



Fig. 5. Location of lateral thyroid rudiment of *Typhlomolge* 6. I., first gill arch; II., second gill arch; *B*, basibranchial; $G_1$, blade of first gill; $G_2$, blade of second gill; *H*, hyoid; *L*, lymph space; *M*, cavity of mouth; *O*, operculum; *P*, pericardium; *T*, thyroid.

In *Typhlomolge* 2, an animal of 77.5 mm. total length and 43.6 mm. body length, only the lateral rudiments are present. Their location is the same as that of the lateral rudiments described above. Instead of being broken up into several separate cell masses, each of them has the shape of one continuous epithelial cell tube possessing a narrow lumen (Fig. 6).

In *Typhlomolge* 5, an animal of 66.0 mm. total length and 36.5 mm. body length, only one lateral rudiment, the left one, is present. It is located near the connection between the ceratobranchial and epibranchial of the first gill arch, median to it and anterior to the location of a normal thyroid of *Ambystoma opacum*. It is composed of a small number of vesicles (Fig. 7) which, instead of being arranged in an antero-posterior row as in the other specimens, are crowded together in one place. The vesicles have a distinct epithelial lining and are hollow; they do not contain colloid. In addition to this rudiment, *Typhlomolge* 5 possesses another one of similar structure, located on the same side but more median. It is attached to the left side of the muscle just ventral to the basibranchial and just anterior to where the first gill arch connects with the basibranchial. Thus it has very nearly the same location as the median rudiment of other animals, but is displaced slightly to the left side. Apparently the primary median rudiment of this animal split into two rudiments; one of them, the left one, moved into its normal position. The right one not only failed to do so, but was dragged along a short distance by the left rudiment before complete separation was

FIG. 6. Left lateral thyroid rudiment of *Typhlomolge* 2. × 340.

FIG. 7. Left thyroid rudiment of *Typhlomolge* 5. × 340.

accomplished, and thus was dislocated from its primary median position to wards the left side.

The 6 specimens described so far died on the way from Texas to New York, shortly after they had been removed from the caves. The seventh animal, *Typhlomolge* I, a specimen of 97.5 mm., died after it had been kept alive in the laboratory for 14 months. The largest part of this time (12 months) it lived at a temperature of 15° C. and in darkness; for the last two months it was kept in an aquarium stocked with plants, small crustaceans and young tadpoles, in daylight and at a temperature of approximately 22 to 25° C. The sections of this specimen are greatly torn and I am not sure that our inability to find a median and left lateral rudiment is due to the absence of these organs and not to the poor condition of the sections. On the right side, however, a lateral rudiment is present. It is located near the lymph space, anterior and dorsal to the location of the main portion of the thyroid of *Ambystoma opacum* and resembles more closely a thyroid structure than the rudiments of the specimens described previously. It consists of a small number of hollow vesicles which compose an elongate, egg-shaped organ possessing a connective tissue capsule and hence impressing one as a distinct and individual organ. The walls of the vesicles are of epithelial character; but no colloid is contained in the lumen of the vesicles (Fig. 8).

Summary: Although *Typhlomolge*, in its advanced stages, does not possess an organ resembling the normal thyroid of a salamander, epithelial structures are found which indicate that in the young embryo of this animal the thyroid rudiment forms in a similar manner as in other amphibians. This rudiment, however, for some reason, fails to develop into a thyroid. In some animals, development ceases after the median epithelial outgrowth has separated from the pharyngeal epithelium and the rudiment remains a single vesicle. In other cases it may partly split up into lateral portions which, as in other salamanders, move in a posterior direction and, in some instances, may approach closely the lymph space; but they never reach the anterior cardinal vein. The unsplit part of the median rudiment may retain its median position and primitive vesicular structure; the lateral portions may either develop into a continuous epithelial tube or may break up into a series of solid or hollow cell masses. In some animals no median rudiment is found; if this condition develops in consequence of a complete splitting-up of the median rudiment or of later degeneration of the median rudiment, can be decided only by studying the embryology of this animal. In one specimen no vestiges of the thyroid are present at all. If Emerson's and Swingle's statements are not due to an oversight, on their part, of the inconspicuous epithelial structures, there are now 6 specimens of *Typhlomolge* known which did not possess any vestiges of the thyroid. One was described by Emerson, 3 by Swingle, one was found previously by me and the sixth animal is the one described in this paper. Either no thyroid rudiments developed in these six animals, or they were reabsorbed shortly after they had developed.

Fig. 8. Right thyroid rudiment of *Typhlomolge* I. ×. 340.

It is certain that the thyroid rudiments of *Typhlomolge* which do persist retain permanently a primitive epithelial structure and fail to develop, among many other structures of a normal thyroid, a venous rete and the colloid.

## Other Endocrine Organs.

It may be briefly mentioned that the thymus glands, the hypophysis, and the postbranchial body were found to be present in every specimen. Like other salamanders, *Typhlomolge* possesses 3 pairs of thymus glands; in one animal they were found fused into two large glands, one on each side. This condition is frequently met with in adult salamanders.

The postbranchial body, although, on a whole, it resembles this organ in other salamanders, shows certain peculiarities (see Baldwin's paper [1] for a description of this organ). Its structure is very similar to that found in *A. opacum;* in particular, it is found only on the left side. It is an epithelial structure of the shape of a tube possessing, in places, epithelial diverticula. A lumen is frequently absent, while in *A. opacum* and other Ambystomidæ this organ possesses often a very condiserable lumen. The cephalic end of the organ is located in the pharyngeal epithelium with which it connects near the place where in Ambystomidæ the *Aditus laryngeus* is situated. In the Ambystomidæ the posterior end of the organ is often very large as compared to the thin duct-like anterior end and is located on the left side of the pericardium, posterior to the fourth aortic arch. Frequently it is closely attached to the pericardium and posterior wall of the fourth aortic arch. In *Typhlomolge* the fourth aortic arch is missing; the postbranchial body attaches itself to the third aortic arch. In some animals it reaches back to the heart and is found attached to the pericardium. Its posterior end, however, does not attain the size which this part is found to attain in *Ambystoma.* Moreover, in some specimens the organ remains short, extending backward only to the middle between pharynx and pericardium. In these cases its posterior end becomes attached to the wall of the third gill arch approximately half-way between the pericardium and the entrance of the arch into the gill blade of the third gill. It seems that the postbranchial body of *Typhlomolge,* although it possesses, on the whole, the structure of the normal organ of the Ambystomidæ, shows sometimes signs of developmental inhibition.

The hypophysis was studied only in two animals and only in transverse sections. Like the hypophysis of the Ambystomidæ [2] it is composed of 4 parts, the pars anterior proper, the partes tuberales, the pars intermedia and the pars nervosa. In the pars

[1] Baldwin, F. M., *Jour. Morph.*, 1918, XXX., 605.

[2] Atwell, W. J., *Anat. Record*, 1921, XXII., 373.

anterior, the largest part of the entire organ, the individual tubes are discernible more distinctly than in the Ambystomidæ. They take an antero-posterior course and are arranged parallel to each other (Fig. 9). In the spaces separating the individual tubes,

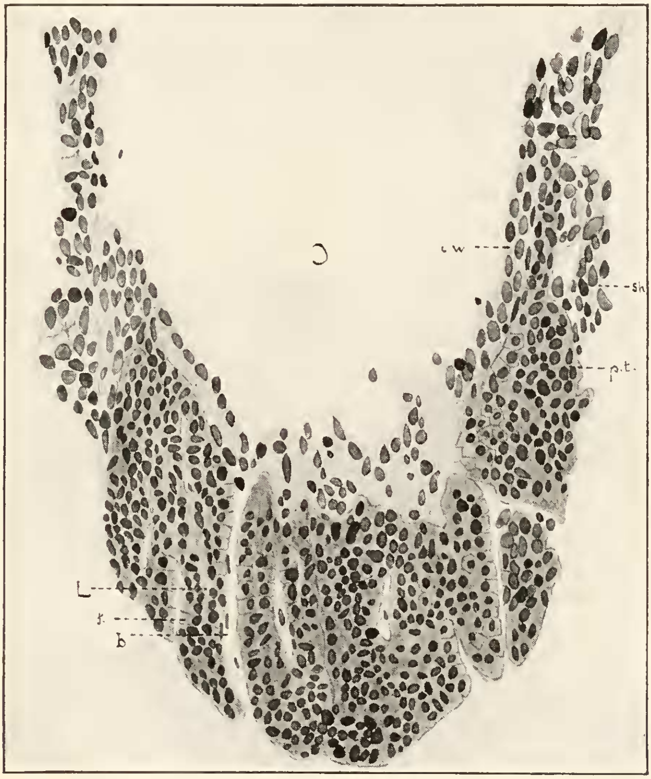



FIG. 9. Transverse section through the hypophysis of *Typhlomolge* 7. *b*, blood vessel; Ɔ-infundibulum; *i.w.*, infundibular wall; *L*, lumen of individual tube; *p.t.*, left pars tuberalis (of the right pars tuberalis this section contains only the connective tissue sheet of the ventral surface). *Sh*, connective tissue sheet. *t*, individual tube of pars anterior. × 340.

large blood vessels are located. Frequently the individual tubes possess a distinct lumen; the nuclei of the cells are located at the distal end of the cell, towards the lumen of the tube. The cell walls are sometimes very distinct. The anterior end of the pars anterior continues into two lateral processes, the partes tuberales, which, like in other salamanders [5, 6, 7], are continuous with the pars anterior and attach themselves to the ventral wall of the infundibulum (Fig. 9). The partes tuberales of *Typhlomolge* are apparently smaller than in the adult *A. opacum* and resemble in size the partes tuberales of a larvæ of *Ambystoma opacum* of about 55 mm. total length and showing no signs of metamorphosis as yet. The pars intermedia of the amphibians cannot well be discriminated from the anterior part in transverse sections. But from such sections as shown in Fig. 10, it would seem that the dorsal part of the pars intermedia is bilobed, the two lobes being separated by a median antero-posterior space. The pars nervosa, as in other salamanders, consists in a thickening of the wall of the infundibulum where the pars intermedia is attached to it (Fig. 10). In comparing the pars nervosa of *Typhlomolge* with that of other salamanders, Haller's description [7] of the pars nervosa of *Proteus anguineus* is of interest. According to this author, the pars nervosa of Proteus is hardly differentiated from the rest of the infundibular wall. In *Typhlomolge* the pars nervosa is not only well-differentiated, but seems to be larger and more sacculated than is the case in *Ambystoma opacum* (Fig. 10). Summarizing


FIG. 10. Transverse section through dorsal regions of hypophysis of *Typhlomolge* 7. *p.i.*, pars intermedia; *p.n.*, pars nervosa. × 340.

the description of the hypophysis of *Typhlomolge*, one may say that it resembles closely the hypophysis of other salamanders. In particular, it does not seem that the hypophysis of this species presents indications of an atrophic state, although, with a larger amount of material at our disposal we might find that the partes tuberales of *Typhlomolge* are of the size of a larval organ.

[6] Haller, B., *Morph. Jahrb.*, 1898, XXV., 30.

[7] Haller, B., *Arch. Mikr. Anat. und Entwicklgsg.*, 1909, LXXIV., 812.

## Discussion.

The factor which led to the inhibition of the thyroid development is unknown. The researches of Leo Adler [8] and Bennett M. Allen [9] showed that extirpation of the hypophysis in amphibians inhibits the development of the thyroid. One could imagine that defective development of the hypophysis might have been the immediate cause of the inhibition of the thyroid development in *Typhlomolge*, but so far, no abnormalities in the structure or mutual relations of the various parts of the hypophysis have been discovered, which could account for the thyroid atrophy of *Typhlomolge*—a phenomenon singular in the vertebrates.

It is not known whether the atrophy of the thyroid of *Typhlomolge* is only one of the results caused by certain factors which lead to the inhibition of the general development of this animal, or whether the inhibition of the thyroid was primary to other developmental inhibitions. As pointed out in previous papers [10] the athyroidism of this species possesses special interest, because in the same species metamorphosis also is suppressed. I have assumed, in a purely tentative manner and in order to obtain a basis for further experiments, that the latter phenomenon is the direct result of the lack of the thyroid. In the absence of adequate experiments and in the light of the well known fact that extirpation of the thyroid inhibits the amphibian metamorphosis [11], [12], this explanation still seems to be the most feasible one.

Swingle,[13] in a recently published article, takes occasion to criticise my attitude, outlined above, towards the problem of neoteny in *Typhlomolge*. He has made certain observations confirming the existence of a releasing mechanism in salamanders. Regarding the facts communicated in this article, these surely should be welcome to the writer of the present article, in so far as they led Swingle to exactly the same conclusion as that at which I arrived as early as 1919. But certain statements made in this paper are apt to give rise to misunderstandings. To prevent these a discussion of Swingle's paper seems desirable.

[8] Adler, L., *Arch. Entwcklgsmech. d. Org.*, 1914, XXXIX., 21.

[9] Allen, B. M., *Biol. Bull.*, 1917, XXXII., 117.

[10] Uhlenhuth, E., *Endocrinology*, 1922, VI., 102.

[11] Allen, B. M., *Science*, 1916, N. S. XLIV., 755.

[12] Hoskins, E. R., and Hoskins, M. M., *Jour. Exp. Zoöl.*, 1919, XXIX., 1.

[13] Swingle, W. W., *Jour. Exp. Zoöl.*, 1922, XXXVI., 397.

Throughout his paper Swingle attempts to create in the reader's mind the impression that in my previous work I have laid too much one-sided emphasis on the thyroid gland as the only organ potent in the amphibian metamorphosis. This attitude is perplexing in view of the circumstance that I have been able to disclose facts demonstrating the existence of a releasing mechanism outside of the thyroid and in view of the other circumstance that Swingle received the discovery of this "releasing mechanism" when communicated by the writer in 1919[14] in the following way[15] (p. 600): "Uhlenhuth, while accepting the conclusions stated regarding the relation of iodine to amphibian metamorphosis, thinks that still another substance is needed to cause the thyroid gland to excrete the iodine necessary for metamorphosis. This hypothetical factor he terms excretor substance and thinks that it is evolved during the growth processes of the organism. The assumption of an excretor substance obscures rather than clarifies the already sufficiently complicated problem of amphibian metamorphosis."

As to Swingle's criticism regarding the omission, on my part, of the possible defectiveness of the releasing mechanism in the explanation of the neoteny of *Typhlomolge*, it should be pointed out that the writer of this article has, reluctantly, refrained from suggesting this possibility, because no experiments suggesting it have been—or are today—available. As to the actual interpretation of the neoteny of *Typhlomolge* and as to my attitude[16] towards Jensen's[17] experiments which showed that adult *Proteus* and *Necturus* do not metamorphose upon thyroid administration, the following statements may be made: (1) the interpretation of this phenomenon as given in my previous papers[16] does not form an integral part of the theory of a releasing mechanism; (2) the most pertinent problem in regard to the neoteny of *Typhlomolge* was the question whether or not this animal possesses a thyroid gland proper, a question to the solution of which Swingle has contributed nothing, as will become evident from a perusal

[14] Uhlenhuth, E., *Jour. Gen. Phys.*, 1919, I., 473.

[15] Swingle, W. W., *Jour. Gen. Phys.*, 1919, I., 593.

[16] Uhlenhuth, E., *American Naturalist*, 1921, LV., 193.

[17] Jensen, C. O. *Oversigt klg. Danske Vidensk. Forhandl.*, Copenhagen, 1916, No. 3, 251.

of the introduction to this article and of the facts described above; (3) the lack of an effective releasing mechanism may have been the primary cause of the neoteny of *Typhlomolge*, but so far nothing as to this effect can be quoted; (4) the question of whether or not *Typhlomolge* and phsyiologically similar species, such as *Proteus* and *Necturus*, possess, in their present state, the ability to metamorphose upon thyroid administration is a problem entirely aside from the rôle of the releasing mechanism and has been considered so in the writer's previous papers. (It is possible that permanent suppression of the thyroid function over long periods may cause complete loss of the reactivity of the organism. The demonstration of such complete loss, however, does not decide the question as to whether primarily the thyroid ceased to function, in *Typhlomolge*, on account of a defective releasing mechanism); (5) it has not been proved as yet that *Typhlomolge*, *Proteus*, and *Necturus* have completely lost their ability to react to the thyroid hormone. It was merely this point which gave occasion for the criticism of Jensen's work. Jensen [17] exposed only the adult specimens of *Proteus* and *Necturus* to the action of the thyroid hormone. That he did not succeed in enforcing metamorphosis does not necessarily mean that the responsiveness of these animals has been completely lost. In order to show that *Necturus*, *Proteus*, and *Typhlomolge* could not metamorphose even if they were in the possession of a complete and normal thyroid mechanism, the young larvæ or even the parents at the time of development or ripening of the ova and spermatozoa may have to be subjected to thyroid administration. Swingle has merely repeated Jensen's experiments on *Necturus*, without modifying Jensen's technique. Like Jensen, he did not use the young larvæ, but the adult animals. Nowhere in Swingle's paper, however, can there be found any reference to Jensen's experiments on *Necturus*.

The same attitude is met with in Swingle's paper regarding the releasing mechanism. Although no progress beyond the present state of the problem has been accomplished, there is, in Swingle's article, no mention made anywhere of previous work on the same problem.

That the thyroid mechanism of salamanders consists of two physiologically distinct parts was found by the writer of this paper as early as 1919.[14] The discovery of the factor necessary to release the thryoid hormone was summed up in the following statement,[14] (p. 476): "Hence, besides iodine, still another substance is needed in the amphibian metamorphosis; namely the 'excretor substance' which causes the thyroid to excrete the stored-up iodine." Swingle's statement [15] (p. 600), made in 1919 in reference to this work and quoted above, shows that he did not recognize the existence of such a releasing factor.

Since organs of internal secretion or any other organs would manifest themselves physiologically in a manner essentially similar to a substance and since it seemed undesirable to reflect in the term applied to the releasing factor upon any preconceived theory, the term "excretor substance" was replaced later on by the term "releasing factor" [16] (p. 207) and "releasing mechanism" [10] (p. 112), both of them implying merely the function by which this factor manifests itself and which was actually observed.

Since the first communication was made my experiments were continued and it was shown, for 3 different species of salamanders, that in low temperature metamorphosis is greatly retarded in proportion to general growth, while the development of the thyroid gland shows no such retardation. It was concluded from this fact that since the thyroid developed at a normal rate in proportion to general growth the development of the releasing mechanism was retarded. Several papers were published in regard to this problem and the difference between the retardation of the thyroid and the releasing mechanism in response to the same degree of lowered temperature was explained by assuming a lower temperature coefficient for the thyroid than for the releasing mechanism. In 1921 the results of this work were summarized in the following statement [16] (p. 206): "The most conspicuous character in the salamander metamorphosis is the fact that although it certainly is dependent on the thyroid hormone, it does not necessarily take place in larvæ whose thyroid is mature. This can only mean that two factors are required in order to bring about the metamorphosis of salamander larvæ, namely a mature gland and a factor which releases the thyroid hormone from the follicles of the gland."

Further confirmation of the existence of a releasing mechanism has been found in the iodine experiments.[10, 18, 19, 20] Administration of an excess of inorganic iodine does not enforce the metamorphosis of salamander larvæ,[10, 20] yet the elaboration of the colloid is accelerated by iodine feeding. This result was to be expected if the release of the hormone does not depend on the quantity of hormone developed in the follicles of the thyroid but is controlled by a particular releasing mechanism.

The results outlined above were checked also by histological sections of large numbers of thyroids of normal and experimental animals. Although the publication in full of this work has been postponed in order to assure greater completeness, single results have been referred to in various papers and have been demonstrated to colleagues and before meetings. In every case it was found that the elaboraion of the colloid and the excretion of it are two distinct and independent processes, physiologically as well as structurally. Elaboration of normal colloid is frequently met with in cases of inhibition of metamorphosis and in normal larvæ long before metamorphosis, and, in this case, is combined with complete absence of the structures characteristic of the excreting stage of the thyroid. This relation has been interpreted as further testimony in favor of the existence of a releasing mechanism.

I must also refer here to Swingle's criticism of my iodine experiments, since, if correct, it would question the value of these experiments as supporting the theory of the releasing mechanism. My experiments [10, 20] showed that, contrary to anuran larvæ, in the larvæ of salamanders metamorphosis cannot be enforced by the administration of inorganic iodine. The bearing of this fact upon a general theory of the rôle of iodine in the specific effect of the thyroid hormone has been outlined in detail in two previous papers.[10, 20] Swingle's general attitude in his paper tends to create the impression (1) that I have claimed "iodine has nothing to do with the axolotl metamorphosis" [13] (p. 417) and (2) that somewhere in my papers are to be found statements to the effect that organic iodine compounds cannot enforce the metamorphosis of the axolotl and other salamanders.

[18] Uhlenhuth, E., *Jour. Gen. Phys.*, 1922, IV., 319.

[19] Uhlenhuth, E., Biol. Bull., 1921, XLI., 307.

[20] Uhlenhuth, E., Biol. Bull., 1922, XLII., 143.

As to the first point I should like to refer the reader to the following statement,[10] (p. 114) into which my results on the rôle of iodine were summarized: "That iodine if supplied in excess does not produce metamorphosis of salamander larvæ does not mean, according to what has been said above, that it is not necessary in the metamorphosis of salamanders. Very likely if larvæ of salamanders would be raised on an iodine-free diet and kept in iodine-free water, metamorphosis could not take place."

Regarding the second point Swingle quotes against me his own experiments [13, 21] in which he thinks he has shown that 3–5 di-iodo-tyrosine can enforce metamorphosis of thyroidectomized axolotls, and Jensen's experiments (22) with iodized proteins. Neither Swingle's own experiments nor Jensen's experiments referred to have proved that inorganic iodine can be utilized directly by the axolotl tissues to elaborate the thyroid hormone. The facts regarding the influence of inorganic iodine on the axolotl metamorphosis are, however, widely different from what Swingle would like them to be.

In the first place, Jensen has not only not shown that inorganic iodine does enforce metamorphosis of the axolotl, but on the contrary has shown that inorganic iodine as such is ineffective in the axolotl metamorphosis. In one of his papers, Jensen [23] points out that the effectiveness of thyroid preparations in enforcing the axolotl metamorphosis does not correspond to the iodine-content of these preparations. In a personal conversation, Professor C. O. Jensen told me that he had tested the action of inorganic iodine, but found it ineffective in enforcing the axolotl metamorphosis. Jensen's experiments are therefore entirely in accord with my own experiments. Moreover, Professor Jensen's experiences which are well in accord with my own observations may serve as a warning against the reliability of those experiments which resulted in "enforced metamorphosis" of the axolotl. Among Professor Jensen's strains of the European race of the axolotl there were, in the beginning, animals which gave rise to offspring 50 per cent. of which would metamorphose

[21] Swingle, W. W., *Science*, 1922, N. S., LVI., 720.

[22] Jensen, C. O., *Compt. rend. Soc. Biol.*, 1920, LXXXIII., 315; 1921, LXXXIV. 423; 1921, LXXXV., 391.

[23] Jensen, C. O., *Hospitalstidende*, 1920, LXIII., 505.

spontaneously. Early in his work he began to select carefully individuals which produced 100 per cent. neotenous larvæ.

Swingle also quotes the experiments of Huxley and Hogben,[24] and of Hirschler [25] against me. What Huxley and Hogben really found, however, is that inorganic iodine does not enforce the metamorphosis of axolotls. There are still Hirschler's experiments; these are represented by "one" successful experiment. The total number of Hirschler's experiments on inorganic iodine in relation to axolotl metamorphosis is "two." One animal was given an intraperitoneal injection of iodoform; it died before a conclusive result was obtained. The other animal received an injection of iodine dissolved in potassium iodid; it metamorphosed completely. But the animal illustrated, as a control alongside this experimental animal, shows, contrary to the authors claim, distinct signs of metamorphosis, a reduction of the tail fin and instead of the larval gills mere stubs. It seems to me the number of Hirschler's positive experiments will have to be increased before they can be held against the negative experiments of Jensen, Huxley and Hogben, and myself.

As to Huxley's and Hogben's positive results [24] on the larvæ of *Salamander maculosa* and *triton*, quoted by Swingle against me, it should be stated that the method employed in these experiments is such as to permit of no conclusions whatsoever. In the first place, they did not use the first moulting, but the sizes of the gills as an indicator of metamorphosis. The gills may become reduced in size by the action of many factors different from metamorphosis, particularly by starvation. Since strong iodine solutions were used, it is almost certain that contrary to the authors' impression (quantitative measurements of the food intake were not made) the experimental larvæ fed less well than the controls. Secondly, nowhere in Huxley's and Hogben's paper can I find any statement indicating the size and stage of the larvæ at the beginning of the experiment. Yet if the larvæ were in an advanced larval stage any irritation as serious as that caused by iodine solutions would be sure to bring about precocious metamorphosis.

[24] Huxley, J. S., and Hogben, L. T., *Proc. Royal Soc.*, 1922, XCIII., 36.

[25] Hirschler, J., *Arch. Entwcklgsgsmech. d. Orgn.*, 1922, LI., 482.

It is evident that none of the observations according to which inorganic iodine does enforce the metamorphosis of salamanders can be accepted as correct at the present time.

That organic iodine compounds may enforce the metamorphosis of neotenous forms of salamanders has been claimed repeatedly and may be true, although the axolotl used generally in these experiments appears, for reasons stated above, to be an unreliable material. Jensen was the first one who studied, in an extensive manner, the influence of organic iodine-compounds upon the metamorphosis of axolotls. Where he left the problem it is still at the present time. In particular, Jensen deserves the credit for having recognized that the experiments with iodine could not advance the problem unless thyroidectomized larvæ are used. He was the first one who administered organic iodine compounds to thyroidectomized axolotls and stated [26] that thyroxine can be used directly by the organism without the intermediation of the thyroid. Swingle repeated these experiments [13, 21] using 3–5 di-iodo-tyrosine, a substance which Jensen [27] had found ineffective in the normal axolotl. Swingle reports that 3-5 di-iodo-tyrosine does enforce the metamorphosis of thyroidectomized axolotls. Both Jensen's and Swingle's experiments, however, should be taken with caution as far as the successful thyroidectomy is concerned. I am not certain at all that Swingle realizes that an axolotl possesses 4 thyroid glands, two main portions and two accessory ones. He mentions it nowhere and it is likely that only the main portions were extirpated. The accessory thyroid glands of *A. tigrinum* have a tendency to become very large and, after removal of the main portions, may enlarge considerably, so as to cause finally metamorphosis, as I observed in many larvæ of *A. tigrinum*. It is likely that Swingle's "thyroidless" axolotls were in the possession of two developing accessory glands; that an axolotl does not possess accessory glands I would be willing to believe only if sections through the entire region of the lower jaws, throat, and heart could be presented, since dissection, because of the hidden position of these accessory glands, may fail to demonstrate them. If the main thyroids are removed, it takes a long time before the accessories, in the event that they

[26] Jensen, C. O., *Compt. rend. Soc. Biol.*, 1921, LXXXV., 391.

[27] Jensen, C. O., *Compt. rend. Soc. Biol.*, 1920, LXXXIII., 315.

have been small, attain a size and structure capable of producing metamorphosis. But as shown in my iodine experiments, the feeding of iodine would greatly accelerate the elaboration of the hormone and, if the releasing mechanism is set active (which it was in Swingle's specimens, according to his own statements), metamorphosis may occur months before it takes place in the untreated controls. Swingle has observed his animals apparently only for 6 months; it would be important to know whether the untreated "thyroidectomized" animals did not finally metamorphose.

Swingle [13, 21] mentions also that 3–5 di-brom-tyrosine, when fed to thyroidectomized axolotl larvæ, is incapable of enforcing metamorphosis and thinks that this result is contrary to my own views on the rôle of iodine in the amphibian metamorphosis and in the thyroid hormone. Apparently he did not see the following statement, in which my views were summarized [10] (p. 114): "The views elaborated above are in no way contradictory to the fact that nevertheless, in a biological sense, iodine is an important and essential part of the thyroid hormone; if it were possible to substitute the iodine by any other substance without changing the reactivity of the hormone, biologically this would not make iodine less important, for it is the only substance which, by the mechanism actually available to the organisms, can be used in the manufacture of the thyroid hormone. Although chemically bromine or any other halogen may be able to substitute iodine without changing the chemical or even the physiological reactivity of the thyroid hormone, the organism is unable to use bromine, as shown by Swingle, and presumably the other halogens to make thyroid hormone." I have never claimed that the thyroid or any other organ can manufacture the thyroid hormone from bromine. Swingle has not touched, by his experiments, the real problem. This centers around the question whether the finished thyroid hormone could enforce metamorphosis if it contained bromine instead of iodine; Swingle did not employ such a product in his experiments.

As Swingle correctly states, the crux of the problem of thyroid function is now to find the organ or tissue or substance which plays the rôle of a releasing mechanism to the thyroid gland. I have intentionally refrained, in my previous papers, from forming any theories, aside from those directly suggested by the results of my experiments, as to the nature of the releasing mechanism; devoting pages to discussing assumptions and hypotheses does not materially advance the problem. We know, of course, that the hypophysis has something to do with the development and, possibly, with the function of the thyroid. I have made some experiments, to be published shortly, which seem to indicate that some unknown factor is located in the gills, in the absence of which the thryoid, although it develops in a normal manner, remains incapable of releasing the hormone. Swingle mentions one experiment which was intended to test the activity of the hypophysis of a neotenous axolotl by the grafting method. Although ultimately it may turn out that the hypophysis controls, in some way, the releasing mechanism, Swingle has so far contributed nothing to the solution of this problem.

Hence it is very evident that Swingle has not advanced, by a single step, the problem of neoteny and thyroid function beyond the stage at which my own researches left it.

## Summary.

1. Only in one, a sex-mature specimen, among 7 specimens of *Typhlomolge rathbuni*, is the thyroid completely absent; in the other 6 specimens rudiments of the thyroids are present.

2. The thyroid rudiments are undifferentiated epithelial cell masses located along the path of migration of the thyroid, typical for salamanders. They may contain a lumen, but never contain colloid and blood vessels.

3. *Typhlomolge* possesses 3 pairs of thymus glands.

4. The hypophysis is similar to that of other salamanders. But the partes tuberales are perhaps smaller than in the adult *A. opacum* and the pars nervosa is larger.

5. The postbranchial body resembles much that of other salamanders, but sometimes is shorter and lacking a lumen.